Controlling the conformational constraint of peptides to modulate their target affinity

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2019

document version
Publisher's PDF, also known as Version of record

Appendix

Summary

Cells contain a multitude of different proteins and peptides, acting as molecular tools for the most diverse processes and procedures and fulfilling various tasks. Since it is possible to compile peptides chemically and therefore exceed natural peptide-translation, an assortment of opportunities for the design and use of peptides in diverse applications is feasible. This thesis describes some of these applications.

Chapter 1 summarizes the role of peptides in present-day research and their application as protein-protein inhibitors. Within the first part, various possibilities for the stabilization of peptides are described leading to improved binding affinity, cell permeability and protease stability. Next to acting as inhibiting molecules, peptides are also used as molecular probes as pointed out in the second part of the introduction.

Chapter 2 (*Peptidomimetics as synthetic ligands of the protein FtsQ - Inhibition of the protein-protein interaction between FtsB and FtsQ in bacterial cell division*) describes the evolution of a protein-protein interaction (PPI) inhibitor. Previously, a large and dynamic protein complex (divisome) was identified to be crucial for bacterial cell division. Amongst others, two proteins were found to be mainly involved in the division process (FtsB/FtsQ). This project aimed at finding an inhibitor potentially suppressing bacterial cell division based on this PPI. After structural elucidation of the important interacting region of both proteins FtsB and FtsQ via crystallization, a 24-mer of FtsB was identified to be strongly involved in this protein-protein interaction. Consequently, this peptide was synthesized and utilized in another crystallization experiment. The binding mode of this 24-mer peptide was analyzed in detail with aid of the peptide-protein crystal structure and the affinity of this peptide (FtsB-p) was measured via different methods, e.g. fluorescence polarization. Based on this,

different stabilization techniques as introduced in Chapter 1 within FtsB-p were screened to seek for modified peptides with higher target affinity. Different crosslinking positions (α, β, γ, δ and ε), different techniques such as alkene crosslinks, guanidine bridges and cysteine based crosslinks and different linker lengths (n = 6 - 12) were tested. After revealing different effective crosslinking possibilities resulting in higher affinity candidates, combinations of these crosslinking techniques were implemented. Successful reduction of the peptides' size through truncation studies resulted in a peptidomimetic, which still needs to undergo further refinements, biophysical evaluation as well as testing *in vivo*.

Besides from mimicking a known interaction partner and therefore developing an inhibitor for PPIs, peptides can be used to device probes for protein detection and to read out protein-protein interactions. A new molecular design is presented in **Chapter 3** (*Coiled-coil Peptide Beacon: A Tuneable Conformational Switch for Protein Detection*). The well-known and established concept of nucleic acid-based molecular beacons was transferred to a fully peptidic system. Herein, two parallel coiled-coil peptides (f and q) meant to form an intermolecular superhelix were synthesized in varying lengths (fxx and q21) to obtain dimers of different affinities. Due to a fluorophore and a quenching molecule at the C-terminal end of either counterpart fluorescence intensity measurements result in different affinities for the different coiled-coil peptide pairs. To build an intramolecular coiled-coil peptide beacon, a receptor binding ligand sequence was then introduced to connect the helical counterparts via chemical ligation. The closed and the open receptor-bound state of these coiled-coil peptide beacons (fxx-L-q21) could be discriminated by fluorescence intensity. Characterization of the newly designed probes was performed with different assays, such as mass spectrometry, size exclusion chromatography, dynamic light scattering and circular dichroism. A subsequent competition assay additionally proved site specificity of the novel coiled-coil peptide beacons.

The first three chapters dealt with challenging peptide modifications and constructs. On the other hand, it is also possible to answer demanding biological questions with rather simple peptides. This strategy is also crucial in current chemical biology. The use of short synthetic peptides instead of heterologously expressed proteins simplifies the examination of certain molecular issues. In **Chapter 4** (*Plant cysteine oxidases are dioxygenases that directly enable arginyl transferase-catalysed arginylation of N-end rule targets*) native peptides were used as substrates of arginyl transfer RNA transferase leading to answered questions of regulating pathways in plants.

Chapter 5 (*The Therapeutical Potential of PTEN Modulation Targeting strategies from Gene to Protein*) summarizes the development of PTEN modulation as a therapeutic target. Herein, approaches based on all levels of the biological dogma are acknowledged leading to a profound base for general targeting strategies.